sites. Johnson and colleagues conclude that all three proposed mechanisms may have contributed to differences in genomic targeting among the Lys14 paralogs — although Models 1 and 2 could perhaps have been distinguished from one another more clearly by, for example, testing each factor's affinity for sites in which half-sites preferred by one factor were placed in an arrangement preferred by a second factor.

Taken together, the mechanisms of gene network evolution explored in this study may explain the rewiring and diversification of Lys14's regulatory circuitry in *C. albicans*, and may be directly relevant to the organism's ability to survive within a human host. One of the Lys14 paralogs, Lys143, is a critical regulator of white–opaque switching, which impacts host immunological responses, host niche preferences, and perhaps pathogenicity. Previous work by the same group demonstrated that two other paralogs, Lys14 and Lys144, are essential for effective proliferation of *C. albicans* within the mammalian gut and bloodstream, respectively [13]. The *LYS14* story provides a glimpse of how organisms can adapt pre-existing molecular tools to increase regulatory complexity, acquire new genetic functions, and survive in complex novel environments.

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Synaptic Plasticity: Astrocytes Wrap It Up

In the brain, astrocytes dynamically interact with neuronal synapses via fine processes. New data show that, in response to synaptic plasticity stimuli, astrocyte processes rapidly move towards and enwrap active synapses, aiding in the stabilization and maintenance of active connections.

Nicola J. Allen

The ability to rapidly alter the strength of synaptic connections between neurons is thought to be the molecular basis underlying learning and memory. Therefore, identifying the mechanisms that lead to a change in synaptic strength has fundamental implications for understanding brain function. An increase in synaptic strength is often accompanied by structural alterations of the synapse, including an increase in size of the postsynaptic dendritic spine [1]. Larger spines are also more stable, meaning it is more likely that this synaptic connection and hence the memory it encodes will persist in the brain. Alteration of synaptic strength and stability is not only controlled by neurons themselves, but can be regulated by other cells in the brain, including astrocytes. Astrocytes are a class of glial cell that send out fine processes that interact with and ensheath many synapses [2], forming the tripartite synapse structure [3]. In the adult brain each astrocyte occupies a unique non-overlapping domain, and it is estimated that within that domain one astrocyte contacts as many as 140,000 synapses [4,5]. Astrocytes regulate multiple aspects of synaptic function, for example by producing factors that induce new synapses to form during development, through to the release of gliotransmitters that modulate synaptic plasticity in the adult brain [6]. In this issue of *Current Biology*, Bernardinelli and colleagues provide evidence that during the induction of synaptic plasticity, astrocyte processes rapidly respond to increased neuronal activity by extending towards and enwrapping the active synapse, thus aiding in the induction of synaptic plasticity and long-term stability of the potentiated synapse [7].

Previous electron microscopy studies have shown correlations

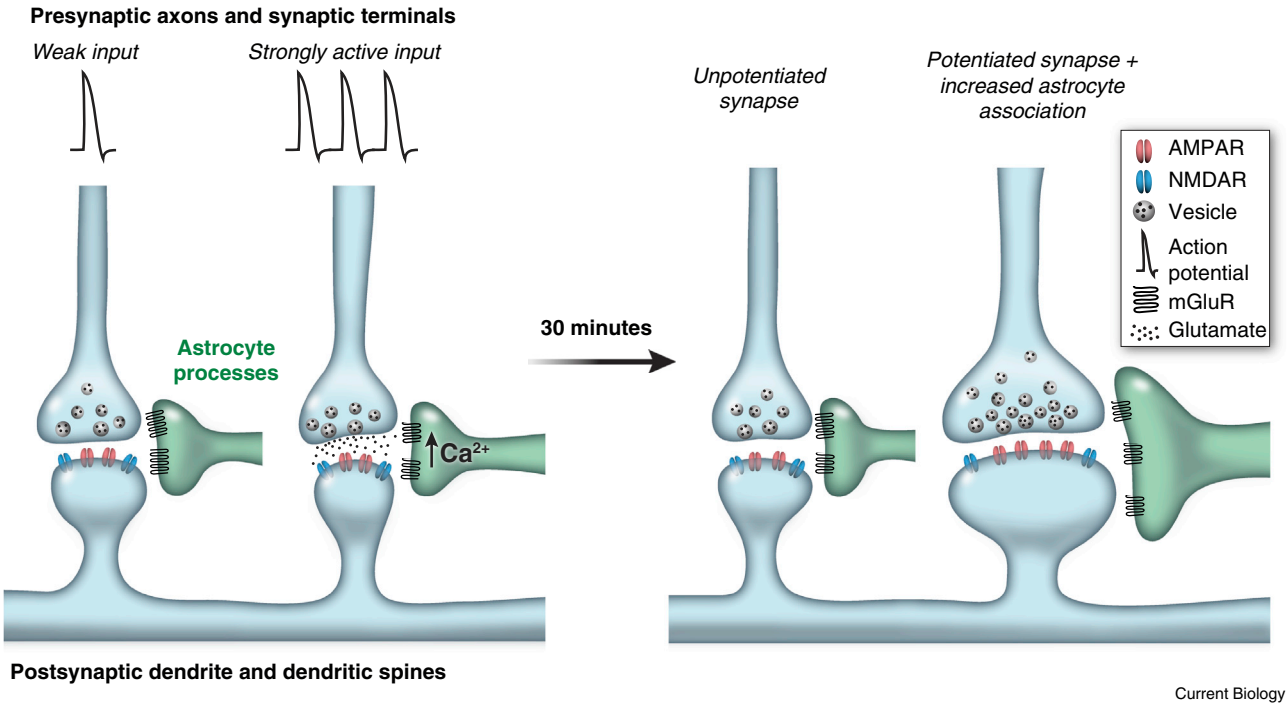

Figure 1. Active synapses attract astrocyte processes, leading to increased enwrapping of the synapse by the astrocyte and synapse stabilization.

A strongly active synapse (right) releases the neurotransmitter glutamate, which is detected by a neighboring astrocyte process via metabotropic glutamate receptors (mGluR). This leads to an increase in calcium concentration in the astrocyte process, and a rapid (minutes) increase in the motility of the astrocyte process towards the active synapse. The dendritic spine increases in volume, and the amount of synapse surface that is contacted by the astrocyte process increases. At a neighboring inactive synapse (left) there is no alteration in the association between the astrocyte process and the synapse, showing that astrocyte–synapse interactions are regulated at the level of individual synapses.

between dendritic spine size and the degree of astrocyte ensheathment, with larger spines more likely to be contacted by an astrocyte process, known as a perisynaptic astrocyte process (PAP) [8]. These static snap-shot studies suggest that astrocytes aid in synaptic stability by preferentially interacting with large, stable synapses. The number of synapses contacted by a PAP also varies between brain regions, and in the hippocampus only 60% of synapses are contacted by a PAP, which has implications for synaptic function and stability [2]. *In vivo* studies in the barrel cortex have demonstrated that 24 hours of whisker stimulation and the associated increase in synaptic activity leads to more wrapping of synapses by PAPs [9]. In addition, live imaging of dendritic spine and PAP dynamics in brain slices demonstrated that PAPs are very motile, and alter their interaction with synapses over minutes [10,11]. There is an inverse correlation between the size of a dendritic spine and the motility of the PAP that interacts with it — PAPs that contact large spines are less motile than PAPs that contact small spines, suggesting increased stability of large synapses may be due to stable PAP contact [12]. This combination of findings has led to the hypothesis that changes in astrocyte–synapse physical interactions contribute to synaptic plasticity and stability, but until now this process had not been investigated in real time.

The current study by Bernardinelli and colleagues tackles this question using live imaging of astrocyte–synapse interactions during the induction of synaptic plasticity, both in hippocampal brain slices and in the barrel cortex *in vivo* [7]. They find that astrocytes rapidly alter the motility and localization of their PAPs during the induction of long-term potentiation (LTP) in the CA1 region of the hippocampus, with a biphasic response profile. For the first ten minutes after LTP is induced there is an initial upregulation of PAP motility towards the active synapse, and this is followed by a decrease in PAP motility 30 minutes after stimulation, and an increased association and enwrapping of the active synapse by the PAP. Importantly, this appears to be specific to active potentiated synapses, as spines that increase in size following the LTP stimulus are more likely to have increased PAP coverage (schematized in Figure 1). The study then dissected out the signaling pathways in astrocytes that lead to increased astrocyte–synapse interactions during LTP. This response required glutamate release from presynaptic terminals and the activation of metabotropic glutamate receptors (mGluRs) on astrocytes, and was dependent on an increase in calcium within the astrocyte. Interestingly, it did not involve activation of the postsynaptic neuron itself, as it occurred when AMPA and NMDA receptors were blocked, suggesting a direct action of presynaptically released glutamate on the astrocyte and no requirement for induction of postsynaptic LTP

in order to induce increased PAP motility.

An interesting finding of this work is that PAPs appear to specifically increase coverage of active, potentiated synapses, suggesting that astrocytes sense and respond to local alterations in neuronal activity and can spatially segregate their responsiveness. The authors executed a series of elegant experiments to test this theory, by expressing Mrg metabotropic receptors in astrocytes, which have no endogenous ligand in the brain and increase intracellular calcium upon activation [13]. They generated a caged form of the Mrg ligand FMRF, so that it could be uncaged by light and used to locally activate Mrg receptors with spatial and temporal control. Using these tools the authors demonstrated that when Mrg receptors were activated on one PAP it caused the PAP to move toward the nearby synapse and increase spine coverage, on a timescale of minutes, while a neighboring PAP that had not been activated remained stationary and did not change its synaptic association. By following the same spines over a 24 hour period it was shown that spines that were contacted by an activated PAP were more likely to still be present after 24 hours than spines next to unactivated PAPs. These results suggest that local activation of metabotropic receptors on individual PAPs leads to PAP movement towards and contact with the active synapse, and only that synapse, thus specifically stabilizing the active connection.

An important extension of this study was to determine whether neuronal activity induced a rapid increase in PAP motility towards active synapses *in vivo*. This was investigated in the barrel cortex, by imaging astrocyte–synapse dynamics during whisker stimulation. When the whisker that innervated the barrel being imaged was stimulated, PAP motility increased within minutes. Importantly, when a whisker that connected to a neighboring barrel was stimulated, this had no effect on PAP motility in the barrel being imaged, demonstrating specificity of the astrocyte response to neuronal activity *in vivo*. PAPs that covered a large part of the spine surface were less motile than PAPs that covered little of the spine. Reimaging of these same spines after four days showed that spines associated with a non-motile PAP were more likely to still be present than spines associated with a motile PAP, as many of these had been eliminated, providing evidence that astrocyte–spine physical interactions stabilize synapses *in vivo*.

This work has important implications for understanding how astrocytes contribute to alterations in synaptic strength, and maintenance of stable synaptic structures. What is the functional advantage for a synapse to have a close physical association with an astrocyte process? The proximity of an astrocyte process to a synapse can profoundly affect many aspects of synaptic transmission, and thus synaptic strength [14]. For example, increased astrocyte–synapse interaction will bring neurotransmitter transporters closer to release sites; alter the ability of neurotransmitter to spillover to neighboring synapses; provide an increased access to energy supplies to active synapses from the astrocyte; and place astrocytes closer to the synapse to allow gliotransmitters to act. Indeed, studies in mutant mice have demonstrated the importance of astrocyte–synapse physical interactions to synaptic function, as mutations that disrupt this interaction have defective plasticity. For example, astrocyte processes and dendritic spines physically interact via EphA4–ephrinA3. In ephrin mutants dendritic spines overgrow and fail to mature, and synaptic plasticity is inhibited [15,16]. In mice lacking the astrocyte gap junction subunit connexin 30, the astrocyte process invades the synapse, bringing glutamate transporters closer to release sites, and alters the ability to induce synaptic plasticity [17].

In summary, an increased interaction of astrocyte processes with dendritic spines in response to strong synaptic activity aids in the long-term maintenance of active synapses, and presumably in memory retention, demonstrating the important contribution of non-neuronal cells to learning and memory.

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